UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES
Antimicrobials Division

May 15, 2006

**MEMORANDUM:**

**Subject:** Efficacy Reg. No. 5813-40 *Pine-Sol Spray 19054*
DP Barcode 326816

**From:** Nancy Whyte, Efficacy Evaluation Team (Acting)
Product Science Branch
Antimicrobials Division (7510C)

May 15, 2006

**To:** Velma Noble/Zenobia Jones
Regulatory Management Branch I, Team 31
Antimicrobials Division (7510C)

**Thru:** Michele E. Wingfield, Chief
Product Science Branch
Antimicrobials Division (7510C)

**Applicant:** The Clorox Company
c/o PS&RC, PO Box 493
Pleasanton, CA 94566-0803

| **Formulation Label:** | **% by wt.** |
|---|---|
| **Active Ingredient(s)** | |
| n-Alkyl (60% $_{C14}$, 30% $C_{16}$, 5% $C_{14}$, 5% $C_{18}$) dimethylbenzyl ammonium chloride | 0.1375% |
| n-Alkyl (68% $C_{12}$,32% $C_{12}$) dimethyl ethylbenzyl ammonium chloride | 0.1375% |
| Other ingredients | 99.7250% |
| **Total** | 100.0000% |

## I. Background:

The product *Pine-Sol Spray 190054* (EPA Reg. No. 5813-41) is a previously registered disinfectant and mildewstat for use on hard, non-porous surfaces in households, commercial, industrial, institutional, food preparation, and animal care environments. The applicant has submitted an amendment to add claims for effectiveness against *Pseudomonas aeruginosa,* Respiratory syncytial virus, Rhinovirus type 37, and Influenza Virus. The label has been re-formatted and includes a significant number of new claims. Efficacy studies were conducted at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121.

The data package contained a letter from the applicant to the Agency dated January 18, 2006, EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), and five studies, MRIDs Nos. 467370-01 thru-05, Statements of No Data Confidentiality Claims for all five studies, and the proposed label.

## II. Use Directions:

The product is designed for disinfecting hard, non-porous surfaces such as appliance exteriors, bed frames, bidets, blinds, cabinets, counter tops, diaper pails, dish racks, door knobs, drinking fountains, faucets, floors, furniture, hand dryer buttons, lamps, laundry hampers, light switches, mattress covers, playground equipment, outdoor and patio furniture, showers, sinks, sneeze guards, telephones, tires, toilet handles and seats, toys, tubs, urinals, vehicles, and walls. The label also indicates that the product may be used on hard, non-porous surfaces including Corian, enamel, fiberglass, glass, glazed ceramic tile, glazed porcelain, glazed tiles, laminated surfaces, synthetic marble, and vinyl. Directions on the proposed label provided the following information regarding use of the product as a disinfectant: Thoroughly cleans surfaces (i.e., applying the product, let the product stand for several minutes, wipe or rinse). Reapply. Let stand for 10 minutes. Wipe.

## III. Agency Standards for Proposed Change:

Disinfectants for Use on Hard Surfaces in Hospitals or Health Care Environments

The effectiveness of disinfectants for use on hard surfaces in hospitals or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for liquid products or water soluble powders) or the AOAC Germicidal spray Products as Disinfectants Method (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 product lots, one of which is at least 60 days old, against *Salmonella enterica,* ATCC 10708, *Staphylococcus aureus* ATCC 6538, and *Pseudomonas aeruginosa* ATCC 15442. To support products labeled as disinfectants, killing on 59 out of 60 carriers is required to support a claim of the product as effective (95% confidence level). These Agency standards are presented in DIS/TSS-1

Virucides

The effectiveness of viral pesticides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products as Disinfectants Method (for spray products) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated

with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least $10^4$ from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virulogical technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

**IV. Summary of Submitted Studies:**

1. **MRID 467370-01 "AOAC Germicidal Spray Method, Test Organism: *Pseudomonas aeruginosa*, ATCC 15442" for *Pine-Sol Spray* 19054, by Sally Nada. Study conducted at ATS Labs. Study completion June 13, 2005. Amended report date--August 13, 2005. Project No. A02950.**

This study was conducted against the organism listed above. Two lots of the product, MJA2005DCDBC-002 and MJA2005CDBC-003, were tested using the method named above as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use. Sixty (60) glass slide carriers were inoculated with 0.01 mL of a 48-54 hour-old suspension of the test organism. The carriers were dried for 30 minutes at 35-37° C at 40% relative humidity. Each carrier was sprayed (3 pumps) with the product at a distance of 6 inches from the carrier surface. The carriers remained exposed to the product for 10 minutes at 20° C at 25°C at 40% relative humidity. Following exposure, the remaining liquid was drained off. Individual carriers were transferred to 20 mL of D/E Broth to neutralize. All subcultures were incubated for 48+/-4 hours at 35-37° C. Following incubation, the subcultures were examined for presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier populations.
**Note:** The initial repost was amended to correct the GLP study number.

2. **MRID 467370-02 "AOAC Germicidal Spray Method, Test Organism: *Pseudomonas aeruginosa*, ATCC 15442" for *Pine-Sol Spray* 19054, by Sally Nada. Study completion October 18, 2005. Project No. A03260.**

This study was conducted against the organism listed above. One lot of the product, MJA2005DCDBC-001 was tested using the method named above as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. The product was received ready-to-use and was at least 60 days old at the time of testing. Sixty (60) glass slide carriers were inoculated with 0.01 mL of a 48-54 hour-old suspension of the test organism. The carriers were dried for 30-40 minutes at 35-37° C at 40% relative humidity. Each carrier was sprayed (3 pumps) with the product at a distance of 6 inches from the carrier surface. The carriers remained exposed to the product for 10 minutes at 20° C at 30% relative humidity. Following exposure, the remaining liquid was drained off. Individual carriers were transferred to 20 mL of D/E Broth to neutralize . All subcultures were incubated for 48+/-4 hours at 35-37° C. The subcultures were stored for 3 days at 2-8°C. Following incubation and storage, the subcultures were examined for presence or absence of visible growth. Controls included those for purity, sterility, viability, neutralization confirmation, and carrier population.

3. **MRID 467370-03 "Virucidal Efficacy of a Disinfectant for Use on Inamlnate Environmental Surfaces, Virus: Respiratory Syncytial Virus" for Pine-Sol Spray 19054, by Mary J. Miller. Study conducted at ATS Labs. Nada. Study completion July 14, 2005. Amended report date—August 10, 2005. Project Number A02955.**

This study was conducted against Respiratory Syncytial Virus, ATCC VR-26, Strain Long, using Hep-2 cell (human carcinoma, ATCC CCL-23, propagated in-house) Two lots, MJA2005CDBC-002 and -003 of the product were tested according to ATS Labs Protocol No CX070510005 RSV (copy not provided). The product was received ready-to-use. The stock virus culture was adjusted to contain 1% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 18.1°C at 58% relative humidity for 20 minutes. For each lot of product, separate dried virus films were sprayed (3 pumps) with the product at a distance of 6 inches from the carrier surface. The carriers remained exposed to the product for 10 minutes at 18.1°C. Following exposure, the plates were scraped with a cell scraper to resuspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Minimum Essential Medium supplemented with 2% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, 2.5 µg/mL amphotericin B, and 1.0 mM L-glutamine. Following titration, the $10^{-2}$ and $10^{-3}$ dilutions were passed through individual Sephadex columns. Hep-2 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The columns were incubated at 36-38°C in a humidified atmosphere of 5-7% $CO_2$ and scored periodically for 8 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman-Karber.

**Note:** The initial report was amended to correct the GLP study number.

**Note:** Protocol deviation/amendments reported in the study were reviewed and found to be acceptable.

4. **MRID 467370-04 "Virucidal Efficacy of a Disinfectant for Use on Inamlnate Environmental Surfaces, Virus: Rhinovirus Type 37" for Pine-Sol Spray 19054, by Mary J. Miller. Study conducted at ATS Labs. Study completion July 14, 2005. Amended report date—August 10, 2005. Project Number A02954.**

This study was conducted against Rhinovirus Type 37 ATCC-VR, Strain 151-11 using MRC-cells (human embryonic lung cells ATCC CCL-171, propagated in-house). Two lots, MJA2005CEBC-002 and -003 of the product were tested according to ATS Labs Protocol No CX07051005 RHV (copy not provided) .The product was received ready-to-use. The stock virus culture was adjusted to contain 1% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 18.1°C at 58% relative humidity for 20 minutes. For each lot of product, separate dried virus films were sprayed (3 pumps) with the product at a distance of 6 inches from the carrier surface. The carriers remained exposed to the product for 10 minutes at 18.1°C. Following exposure, the plates were scraped with a cell scraper to resuspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Minimum Essential Medium supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Following titration, the $10^{-2}$ dilution

was passed through an individual Sephadex column. MRC-5 cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilution. The cultures were incubated at 31-35˚C in a humidified atmosphere of 5-7% $CO_2$, and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus counts, cytotoxicity, and viability. Controls included those for dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman-Karber.
**Note:** The initial report was amended to correct the GLP study number and typographical error.

5. **MRID 467370-05 "Virucidal Efficacy of a Disinfectant for Use on Inaminate Environmental Surfaces, Virus: Influenza A virus" for Pine-Sol Spray 19054, by Mary J. Miller. Study conducted at ATS Labs. Study completion June 21, 2005. Amended report date—August 10, 2005. Project Number A02957.**

This study was conducted against Influenza A Virus (ATCC VR-544, Strain Hong Kong, using RMK cells (Rhesus monkey kidney cells, originally obtained from Ironed Laboratories, Inc maintained in-house) as the host system . Two lots, MJA2005CEBC-002 and -003 of the product were tested according to ATS Labs Protocol No CX07051005 FLUA (copy not provided) .The product was received ready-to-use. The stock virus culture contained 1% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried at 20˚C at 44% relative humidity for 20 minutes. For each lot of product, separate dried virus films were sprayed (3 pumps) with the product at a distance of 6 inches from the carrier surface. The carriers remained exposed to the product for 10 minutes at 20.0˚C. Following exposure, the plates were scraped with a cell scraper to resuspend the contents. The virus-disinfectant mixture was passed through a Sephadex column, and diluted serially in Minimum Essential Medium supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. Following titration, the $10^{-2}$ dilution was passed through an individual Sephadex column. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilution. The cultures were incubated at 36-38˚C in a humidified atmosphere of 5-7% $CO_2$, and scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus counts, cytotoxicity, and viability. Controls included those for dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman-Karber.
**Note:** The initial report was amended to correct the GLP study number

Results are found in the tables on the following pages:

| MRID Number | Organism | Number exhibiting growth/ Total Number Tested | | | Carrier Population |
|---|---|---|---|---|---|
| | | Lot No. MJA2005 CDSD-002 | Lot No. MJA2005 CDBD-003 | Lot No. MJA2005 CDBD-004 | |
| | | | | | |
| 467370-01 | *Pseudomonas aeruginosa* | 0/60 | 0/60 | | $7.3 \times 10^5$ |
| 467370-02 | *Pseudomonas aeruginosa* | ----- | ---- | 0./60 | $5.7 \times 10^5$ |

| MRID Number | Organism | Results | | | Dried Virus Control ($TCID_{50}$/0.1 mL |
|---|---|---|---|---|---|
| | | | Lot No. MJA2005 CDBC-2 | Lot No. MJA2005 CDBC-03 | |
| 467370-03 | Respiratory Syncytial Virus | $10^{-1}$ dilution | Cytotoxicity | Cytotoxity | $10^{4.5}$ |
| | | $10^{-2}$ to $10^{-7}$ dilutions | Complete Inactivation | Complete inactivation | |
| | | $TCID_{50}$/0.1 mL | $\leq 10^{1.5}$ | $\leq 10^{1.5}$ | |
| | | Log reduction | >3 $\log_{10}$ | >3 $\log_{10}$ | |
| 467370-04 | Rhinovirus Type 37 | $10^{-1}$ dilution | Cytotoxicity | Cytotoxicity | $10^{5.0}$ |
| | | $10^{-2}$ to $10^{-7}$ dilutions | Complete Inactivation | Complete inactivation | |
| | | $TCID_{50}$/0.1 mL | $\leq 10^{1.5}$ | $\leq 10^{1.5}$ | |
| | | Log reduction | >3.5 $\log_{10}$ | >3.5 $\log_{10}$ | |
| 467370-05 | Influenza A Virus | $10^{-1}$ to $10^{-2}$ dilutions | Cytotoxicity | Cytotoxicity | $10^{6.0}$ |
| | | $10^{-3}$ to $10^{-8}$ dilutions | Complete Inactivation | Complete inactivation | |
| | | $TCID_{50}$/0.1 mL | $\leq 10^{2.5}$ | $\leq 10^{2.5}$ | |
| | | Log reduction | >3.5 $\log_{10}$ | >3.5 $\log_{10}$ | |

## V. Conclusions:

1. The submitted efficacy data (MRID Nos. 467370-01 and -02) support the use of the product *Pine–Sol Spray 19054* as a disinfectant against *Pseudomonas aeruginosa* on hard, non-porous surfaces for a contact time of 10 minutes. Killing was observed in the subcultures of all carriers tested against the required number of product lots. At least one of the product lots tested (Lot No. MJA2005CDBC-001) was at least 60 days old at the time
of testing . Carrier population counts were at least $10^4$. Neutralization confirmation testing showed positive growth of the microorganism. The viability controls were positive for growth. Purity controls were reported as pure. The sterility controls did not show growth, hard, non-porous surfaces for a contact time of 10 minutes

2. The submitted efficacy data support the use of the product *Pine–Sol Spray 19054*

as a disinfectant with virucidal activity against the following microorganisms on hard, non-porous surfaces in the presence of a 1% organic soil load for a contact time of 10 minutes.

| | |
|---|---|
| Respiratory syncytial virus | MRID No. 467370-03 |
| Rhinovirus Type 37 | MRID No. 467370-04 |
| Influenza A virus | MRID No. 467370-05 |

Recoverable virus titers of at least $10^4$ were achieved. In studies against Respiratory syncytial virus and Rhinovirus Type 37, cytotoxicity was observed in the $10^1$ dilutions. Complete inactivation (no growth) was indicated in all higher dilutions tested. At least a 3-log reduction in titer was demonstrated beyond the cytotoxic level.

## VI. Labelling:

1. The proposed label claims that the product, *Pine-Sol Spray 19054*, is an effective disinfectant on hard, non-porous surfaces against the following microorganisms in the presence of a 5% organic soil load for a contact time of 10 minutes at full strength.

   *Pseudomonas aeruginosa*
   Respiratory syncytial virus
   Rhinovirus Type 37
   Influenza A virus

   Data provide by the applicant **do not support** these claims. Testing was not conducted in the presence of a moderate soil load. The directions on page 5 of the proposed label (1st item, Pesticide Claims section) must be amended to delete the text "in the presence of a 5% organic soil load". Efficacy has been demonstrated for pre-cleaned surfaces.

2. Testing was not conducted in the presence of a moderate soil load; therefore, all directions to disinfect must include a pre-cleaning statement and to remove excess soil from heavily soiled areas. The directions to disinfect [on page 3 of the proposed label] must be revised as follows:

   - Within the first bullet of test, remove the parentheses from "remove excess dirt first in heavily soiled areas".
   - Within the second bullet of text, insert a statement to pre-clean surfaces thoroughly.

3. The proposed label [see pages 4 (left column; 18th item) and 6 on the proposed label] indicates that the product may be used on fiberglass surfaces. Fiberglass is a porous surface. Please delete these general references to fiberglass. You may indicate on the product label that the product can be used on fiberglass bathtubs or sealed fiberglass surfaces.

4. Please make the following changes to the proposed label, as appropriate:

- On Page 3, delete all claims regarding disinfection. These claims should be included in the Pesticidal Claims section of the label.

- On page 3, delete claims such as "fastest", "nothing's tougher", and "most powerful".

- On page 3 (left column 19th item) delete the optional word "[cleaning]". The word does not make sense in this specific marketing claim.

- On page 4 (left column, 29th item) delete the optional text "[in presence of soil load]." Efficacy testing was not conditioned in the presence of a moderate soill load.

- On page 4 (right column, 18th item), delete the word "bugs". This word is not an appropriate use of the word, nor is it a reference to germs.

- On page 5 (left column, 1st term), change "nonpourous" to read "nonporous".

- On page 5 (3rd item, Pesticidal Claims section) change "bathroon" to read "bathroom".

- On page 5 (13th item, Aesthetic Claims section), delete the claim recommending use of Tilex for mildew problems. This product, *Pine-Sol Spray 19054*, is a mildewstat.

- On Page 5 (13th item, Aesthetic Claims section), delete "Not for Household Use". In all other places, the label promotes use of the product in household Environments.

- On page 6, change "Outdoor –or- Patio Furniture" to read "Outdoor-or-Patio Furniture (except cushions and wood frames